Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

A Mitochondrial Targeting Mn(II)-terpyridine Complex for Two-Photon Photodynamic Therapy

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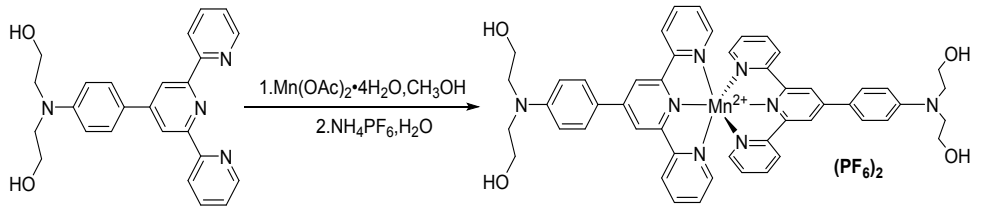
1. Materials and Methods

All the reagents were obtained commercially and used without purification. MitoTracker Deep Red (MTDR) and Lyso Tracker Red (LTR) were bought from Sigma-Aldrich. The ESI-MS was obtained using an Agilent 1290-6545 LC/MS system. Elemental analysis was acquired with Heraeus CHN-O Rapid analytical instrument. UV-vis spectra were recorded on a UV-265 spectrophotometer.

2. Synthesis of MTP



TPYOH $[Mn(TPYOH)_2](PF_6)_2$

Scheme S1. Synthetic route for MTP.

Synthetic of **MTP** (Scheme S1):

4′-p-N,N-Bis(2-hydroxyethyl)aminobenzyl-2,2′:6′,2″-terpyridine (TPYOH) was synthesized according to the previous literature[1]. $Mn(CH_3COO)_2{\cdot}4H_2O$ (184 mg, 0.75 mmol) and TPYOH (618 mg, 1.5 mmol) were dissolved in 30 mL of CH_3OH and refluxed with stirring for 5 h, then the solvent was removed under reduced pressure to obtain bright yellow solid. The solid was then dissolved in water (30 mL), and an excess of saturated aqueous NH_4PF_6 (300 mg) was added. After string for 30 min, the bright yellow precipitate was collected by filtration, washed with water (3 × 20 mL), ethanol (3 × 10 mL), and ethyl diether (10 mL), and then dried under vacuum. Yield: 611.1 mg (76.2%). Elemental analysis for $C_{50}H_{48}F_{12}MnN_8O_4P_2$, calcd: C, 51.34; H, 4.14; N, 9.58; found: C, 51.21; H, 4.03; N, 9.75. ESI-MS (+p): m/z = 440.1574 was assigned as $[Mn(TPYOH)_2]^{2+}$. Red lamellar crystals suitable for X-ray crystallography were obtained by slow evaporation of **MTP** solution in acetone and tert-butanol.

3. Single Crystal Structure Analysis:

X-ray crystallography data were collected using a Bruker APEX CCD X-ray diffractometer with Mo Kα radiation (k = 0.71073 Å) at room temperature, and the data were processed using the Bruker SAINT and SADABS software packages.

The structure was solved by direct method and refined by full matrix least squares of F^2 using the SHELXTL-2018 program. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were included in idealised positions using the riding model. CCDC: 1957693.

4. Emission measurements

One-photon fluorescence spectra were obtained using a HITACHI F-7000 spectrofluorimeter equipped with a 750W Xe lamp. The concentration of sample was 1 × 10^{-5} mol/L. The fluorescence quantum yield (Φ) was determined using quinine sulfate (in 1 mol/L H_2SO_4, Φ = 0.54) as the reference. Quantum yield was corrected as following[2]:

$$\Phi_s = \Phi_r(\frac{D_S}{D_r})(\frac{A_r}{A_s})(\frac{n^2{}_s}{n^2{}_r})$$

Where the s and r indices designate the test sample and reference sample, respectively, A is the absorbance at the excitation wavelength, and n is the refractive index of the solvent used, and D is the integrated area under the corrected emission spectrum.

Two-photon absorption (TPA) cross-section of the sample was obtained by the two-photon excited fluorescence (TPEF) method (using fluorescein as reference) with femtosecond laser pulse and a Ti:sapphire system (680-1080 nm, 80 MHz, 140 fs) as the light source. The concentration of the sample solution was 1 × 10^{-3} mol/L. The experimental fluorescence excitation and detection conditions are conducted with negligible reabsorption processes, which can affect TPA measurements.

$$\delta = \delta_{ref}(\frac{\Phi_{ref}}{\Phi})(\frac{C_{ref}}{C})(\frac{n_{ref}}{n})(\frac{F}{F})$$

Here, the subscripts *ref* stands for the reference molecule. δ is the two-photon absorption cross-section value, C is the concentration of solution, n is the refractive index of the solution, F is the integrated area of the detected two-photon-induced fluorescence signal, and Φ is the fluorescence quantum yield.

5.Quantification of singlet oxygen generation

Singlet oxygen generation studies were carried out with a light source xenon

lamp (400-700 nm, 150 mW·cm^{-2}, model 3767) on an Oriel optical bench (model 11200) with a grating monochromator (model 77250). Quantum yield for singlet oxygen generation in DMSO was determined by monitoring the photoxidation of 1,3-diphenylisobenzofuran (DPBF) sensitized by the **MTP**. DPBF is a convenient acceptor because it absorbs in a region of dye transparency and rapidly scavenges singlet oxygen to give colorless products. This reaction occurs with little or no physical quenching. To evaluate whether the Mn could genrate $^1O_2$, used DPBF (10 μM) as the $^1O_2$ indicator under cell-free conditions. The air-saturated aqueous solution of **MTP** (10 μM) and DPBF (10 μM) were irradiated with a solar simulator in a time interval from 0 to 35 s, and the variation of the absorbance for DPBF was monitored at 410 nm. The quantum yield of singlet oxygen generation (Φ [$^1O_2$]) was calculated by a relative method using optically matched solutions and comparing the quantum yield of photooxidation of DPBF sensitized by the dye of interest to the quantum yield of MB (Φ [$^1O_2$] = 0.52) sensitized DPBF photooxidation as the reference. The quantum yield calculation method is referred to the previous article[3].

6. Cell culture

The human non-small-cell lung cancer cell line (A-549), the human cervix carcinoma cell line (HeLa) and Normal Human hepatocytes cell line (LO2) were seeded in six-well plates at a density of 1×10^5 cells per well and grown for 96 h used DMEM nutrient solution. For cytotoxicity assays and live-cell imaging, cell cultures were incubated with **MTP** solution (5 μM) and maintained at 37ºC under 5% CO_2/95% air for 2 h. The cells were then washed with PBS (3 × 3 mL per well), and PBS (3 mL) was added to each well.

7. In Vitro Cytotoxity Assay.

The cytotoxicity of **MTP** was determined using the MTT assay, as described by Carmichael et al[4], which makes use of the conversion of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to a purple formazan product by the mitochondrial dehydrogenase of viable cells. Detailly, the cells were seeded in a 96-well plate at a density of 5000 cells per well and incubated with 100 μL culture medium containing a series of doses of the sample at 37°C for 48 h. After the incubation, the culture medium in each well was removed and

the cells were washed three times with PBS. 20 μL of MTT solution (5 mg/mL) was added to each well and cultured for another 2 h. The supernatant was discarded and then 100 μL of DMSO was added to each well. The values of the plate were observed on a microplate reader at 570 nm (Safire, Tecan). The results were expressed as the viable percentage of cells after various treatments relative to the control cells without any treatment.

The cell viability (%) was calculated according to the following equation used MTT assays: cell viability% = OD_{570}(sample)/OD_{570}(control) ×100, where OD_{570}(sample) represents the optical density of the wells treated with various concentration of the **MTP** and O_{D570}(control) represents that of the wells treated with DMEM + 10% FCS. Three independent trials were conducted, and the averages and standard deviations are reported. The reported percent cell survival values are relative to untreated control cells.

8. Phototoxicity assay in A549 cells and HeLa cells

After incubation of A549 cells and HeLa cells with various concentrations of **MTP** for 12 hours, and following irradiation for a certain time interval with a xenon lamp (400-700 nm, 150 mW·cm^{-2}) to detect cell viability using MTT assay.

9. Live-cell one- and two-photon fluorescence microscopy imaging

The commercial dyes mitochondrial imaging agent Mito Tracker Deep Red FM (MTDR), and the lysosomal imaging agent Cell Lyso Tracker Red (LTR) was used in the co-localization system, the guidelines for use were download from Life Technologies (www.lifetechnologies.com). All images were acquired on Zeiss LSM880 NLO (2+1 with BIG) Confocal Microscope System equipped with objective LD C-Apochromat 63x/1.15 W Corr M27, cell incubator with temperature control resolution ± 0.1°C, 405 nm Diode laser, Argon ion laser (458, 488 and 514 nm), HeNe laser (543 and 594 nm), Rack LSM 880 incl. 633 nm laser, and a Spectra Physics femtosecond Ti: sapphire laser (~4 W at 800 nm) which corresponded to approximately 1% (~40 mW at 800 nm, the output laser pulses have a tunable center wavelength from 690 nm to 1040 nm with pulse duration of 150 < fs and a repetition rate of 80 MHz) average power in the focal plane as the excitation source, with main beam splitter wheel VIS equipped for ROGB lasers/Axio imager beam coupling optics for NLO and 405 nm laser and 8

channels AOTF for simultaneous control of 8 laser lines. A PMT detector ranging from 420 nm to 700 nm for steady-state fluorescence and non-descanned detectors (BiG.2) for the TPEF, were used. Internal photomultiplier tubes were used to collect the signals in 8-bit unsigned 1024 × 1024 pixels at a scan speed of 200 Hz. Images were processed with Zeiss User PC Advanced for LSM system (BLUE).

10. Detection of intracellular ROS.

Intracellular ROS generation was examined by using CellRox® Green Reagent. Cells were treated with **MTP** (1 μM) for 30 min, followed by being illuminated with Xenon lamp for 30 min, then CellRox® Green Reagent (10 μM) was loaded for another 30 min at 37°C. Cells were washed three times with ice-cold PBS, after that fixed with 4% paraformaldehyde for 15 min. Then, after three times PBS washing, ROS production in the cells was immediately monitored by fluorescence microscopy. The mean signal intensities of CellRox® Green Reagent were analyzed with an Image J software according to the previous report[5].

11. Comparative assay of ATP

ATP levels in the supernatant were measured using an ATP assay kit (Beyotime, Jiangsu, China). 3 × 10^5 cells were seeded in a 6-well plate. When the cells grew to a density of about 70%, HeLa Cells were incubated with **MTP** for 10 min and then treated with light irradiation for 0 min, 2 min, 5 min, 10 min. The cells were collected and lysed with ATP detection lysate on ice, then centrifuged at 13000 rpm for 5 min at 4°C, and the supernatant was collected for ATP determination. Dilute the ATP standard solution (0.5 mM) with ATP detection lysate to a concentration of 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM, and then dilute the ATP detection reagent with the ATP detection reagent dilution 10-fold to Prepare an appropriate volume of ATP detection working solution. Add 100 μL of ATP detection working solution to each well of a 96-well plate, and leave it at room temperature for 3-5 min to completely consume the background ATP, thereby reducing the background. Then add 20 μL of sample and standard to the detection well, and then measure the chemiluminescence value on a microplate reader after a 2 s interval. Draw a standard curve for the ATP standard and calculate the ATP concentration in the sample. the protein concentration of each

treatment group was determined using the Bradford protein assay. The intracellular ATP level was normalized by protein content in each sample.

12. Comparative assay of mitochondrial respiratory chain Complex I

Mitochondrial respiratory chain complex I levels in the HeLa cells were measured using a human mitochondrial respiratory chain complex I ELISA Kit (Human MRCC I ELISA Kit). (Jinlin biology science and technology development co., ltd, Nanjing, Jiangsu, China). 3×10^5 cells were seeded in a 6-well plate. When the cells grew to a density of about 70%, HeLa Cells were incubated with **MTP** for 10 min and then treated with light irradiation for 0 min, 2 min. The cells were collected and lysed with PBS solution with 0.1% Triton and 0.1% PMSF on ice, then centrifuged at 13000 rpm for 15 min at 4°C, and the supernatant was collected for complex I determination. Dilute the complex I standard solution (450 ng/L) with complex I standard diluent to a concentration of 300 ng/L, 200ng/L, 100 ng/L, 50ng/L, 25ng/L. Set blank wells separately and testing sample well. Add Sample dilution 25 μL to testing sample well, then add testing sample 25 μL (sample final dilution is 2-fold), add sample to wells, gently mix. After closing plate with Closure plate membrane, incubate for 30 min at 37°C. Then configurate liquid: 30-fold wash solution diluted 30-fold with distilled water and reserve. Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat. And then add HRP-Conjugate reagent 50 μL to each well, except blank well. Incubated for 30 min at 37°C and washed by wash solution. Add Chromogen Solution A 50 μL and Chromogen Solution B 50 μL to each well, evade the light preservation for 15 min at 37°C. Add the Stop Solution 50 μL to each well. Read absorbance at 450 nm. Draw a standard curve for the Complex I standard and calculate the Complex I concentration in the sample. And then normalized the concentration.

13. Detection of ROS induced by Two-Photon Laser

HeLa cells were incubated in 35 mm petri dish with glass bottom. The cells were incubated with **MTP** for 10 min and after the light, adding 5μM CellRox® Green, continue to incubate for 30 minutes. Two-photon confocal microscope was used to scan this area in real time, with a laser wavelength of 770 nm and a voltage of

10% (~4 mW). ROS production in the cells was immediately monitored by fluorescence microscopy.

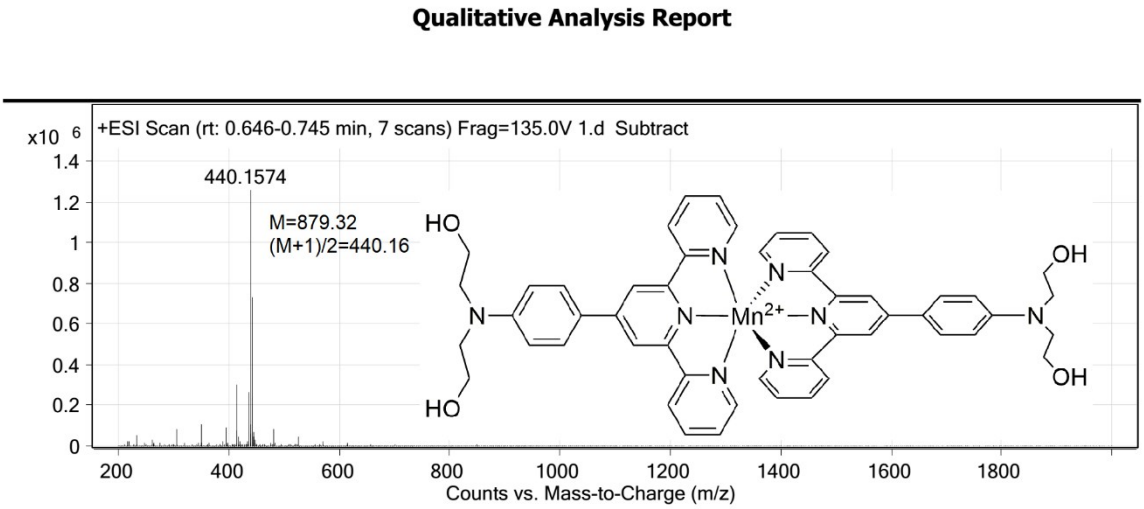


Fig. S1 ESI-MS spectrum of **MTP**.

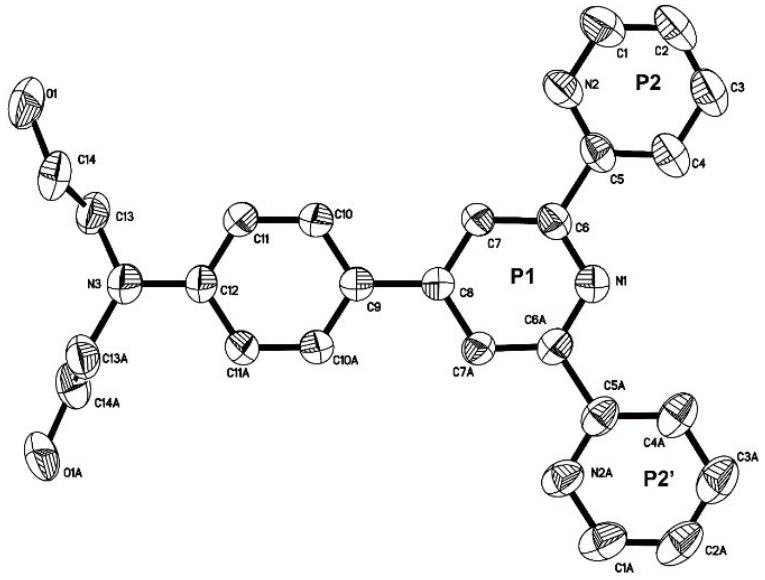


Fig. S2 ORTEP drawing for TPYOH. H atoms were omitted for clarity.

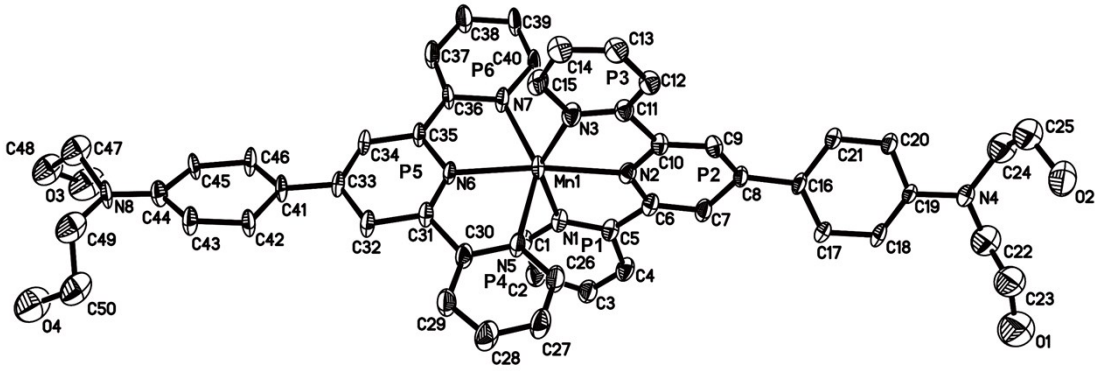


Fig. S3 ORTEP drawing for **MTP**. the anions and H atoms were omitted for clarity.

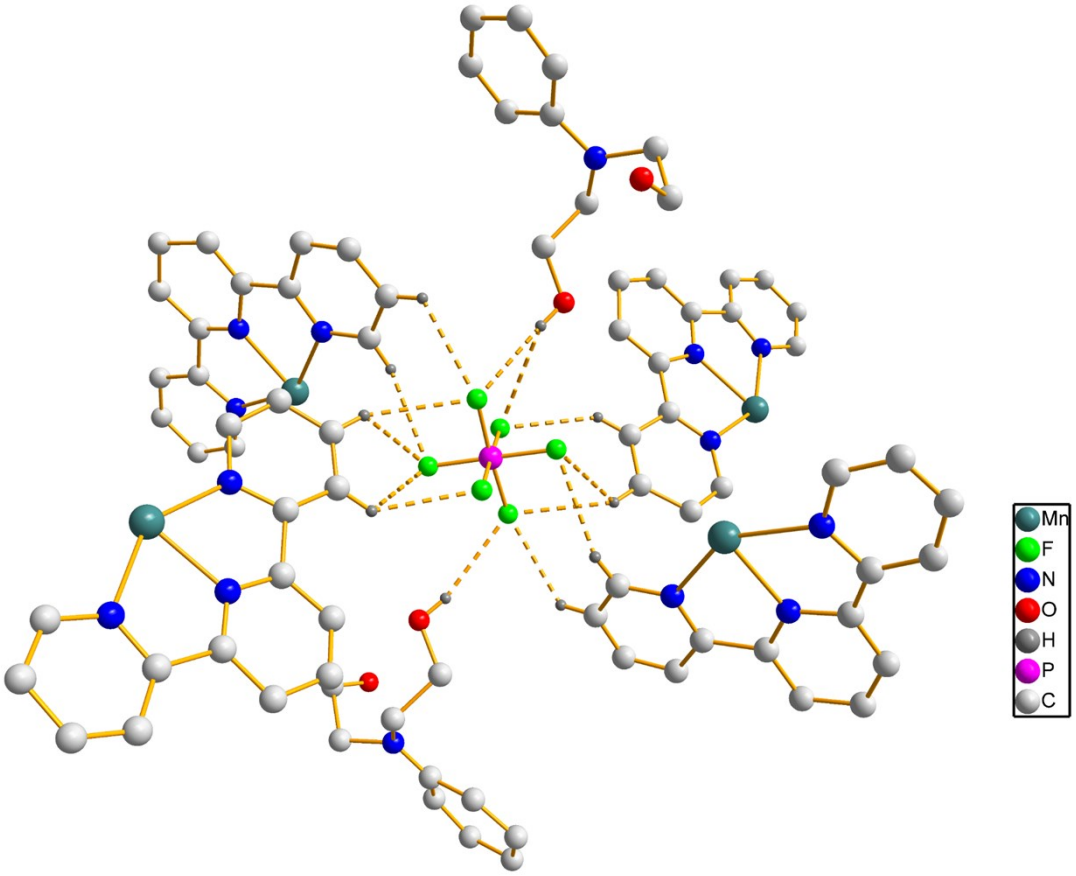


Fig. S4 Diagrams of hydrogen bonding between the PF_6^- and C-H, O-H groups in the **MTP**. Hydrogen atoms are omitted for clarity.

The PF_6^- anion played crucial roles in connecting the cations into 3D networks and all fluorine atoms acted as H-bonding electron donors; each PF_6^- formed hydrogen bonds with the adjacent four cationic cores. The C-H groups from the adjacent pyridine rings and hydroxyethyl groups served as the H-bonding acceptors. The {N,N,N} coordination planes were observed to be nearly perpendicular to each other with dihedral angles of about 85.681°. The $Mn\text{-}N_{lateral}$ bond distance in **MTP** was measured to be about 2.124-2.263 Å (Tables S1-S3) similar to the reported values for Mn^{II}-terpyridine complexes.[6,7] The X-ray crystallography data showed most bond lengths in the coordinating pyridine rings to be shorter than those of the free ligand, and the dihedral angles to be less than those of the free ligand, indicating better coplanarity of the terpyridinyl group and a better conjugated geometric configuration of TPYOH in the complex than in the free ligand.

Table S1. Crystallographic parameters for the Mn(II) complex (**MTP**).

Compound	**MTP**	V / Å3	5013.9(10)
Empirical formula	$C_{50}H_{48}N_8O_4P_2F_{12}Mn$	Z	4
Formula weight	1169.84	D calcd/(g·cm^{-3})	1.550
T [K]	293(2)	F(000)	2396
Crystal system	Triclinic	Absorption coefficient	0.427 mm^{-1}
Space group	P-1	θ range	1.104 ~26.000°
a [Å]	8.5850(11)	Reflections collected	19304
b [Å]	15.9090(18)	independent reflections	19304
c [Å]	37.260(4)	GOF on F^2	1.099
α [°]	97.656(3)	R_1 [I>2σ(I)]	0.1639
β [°]	91.6860(10)	wR_2 [I>2σ(I)]	0.3622
γ [°]	95.702(2)	CCDC	1957693

Table S2. The dihedral angle between pyridines of TPYOH and **MTP**.

Compound	Dihedral angle (°)
TPYOH	P1-P2 (P1-P2') 9.41; P2-P2' 14.48.
MTP	P1-P2 8.38; P2-P3 6.66; P1-P3 9.10; P4-P5 5.20; P5-P6 2.53; P4-P6 6.54.

Table S3. Selected bond lengths and bond angels for TPYOH and **MTP.**

Compound	Bond Length	Bond Angel
TPYOH	N(1)-C(6) 1.333(5) N(1)-C(6)#1 1.333(5) N(2)-C(5) 1.328(6) N(2)-C(1) 1.356(6) N(3)-C(12) 1.394(10) N(3)-C(13') 1.51(3) N(3)-C(13')#1 1.51(3) N(3)-C(13) 1.55(2) N(3)-C(13)#1 1.55(2)	C(6)-N(1)-C(6)#1 117.8(5) C(5)-N(2)-C(1) 117.1(5) C(12)-N(3)-C(13') 117.2(6) C(12)-N(3)-C(13')#1 117.2(6) C(13')-N(3)-C(13')#1 125.6(12) C(12)-N(3)-C(13) 115.7(6) C(13')-N(3)-C(13) 51.8(7) C(13')#1-N(3)-C(13) 102.8(9) C(12)-N(3)-C(13)#1 115.7(6) C(13')-N(3)-C(13)#1 102.8(9) C(13')#1-N(3)-C(13)#1 51.8(7) C(13)-N(3)-C(13)#1 128.6(12) C(14)-O(1)-H(1) 109.5 C(14')-O(1')-H(1') 109.5 N(2)-C(1)-C(2) 122.7(6) N(2)-C(5)-C(4) 121.8(4) N(2)-C(5)-C(6) 116.8(4) C(4)-C(5)-C(6) 121.4(4) N(1)-C(6)-C(7) 122.5(4) N(1)-C(6)-C(5) 116.3(4)
MTP	Mn(1)-N(6) 2.190(12) Mn(1)-N(2) 2.214(12) Mn(1)-N(1) 2.215(14) Mn(1)-N(3) 2.223(13) Mn(1)-N(7) 2.257(13) Mn(1)-N(5) 2.293(13) N(1)-C(5) 1.323(18) N(1)-C(1) 1.337(18) N(2)-C(10) 1.326(18) N(2)-C(6) 1.366(19) N(3)-C(11) 1.274(17) N(3)-C(15) 1.406(19) N(4)-C(19) 1.400(18) N(4)-C(22) 1.476(9) N(4)-C(24) 1.482(9) N(5)-C(26) 1.310(17)	N(6)-Mn(1)-N(2) 172.3(5) N(6)-Mn(1)-N(1) 103.3(5) N(2)-Mn(1)-N(1) 74.2(5) N(6)-Mn(1)-N(3) 111.8(5) N(2)-Mn(1)-N(3) 70.4(5) N(1)-Mn(1)-N(3) 144.6(5) N(6)-Mn(1)-N(7) 70.2(5) N(2)-Mn(1)-N(7) 117.3(5) N(1)-Mn(1)-N(7) 100.0(5) N(3)-Mn(1)-N(7) 95.5(5) N(6)-Mn(1)-N(5) 73.5(5) N(2)-Mn(1)-N(5) 99.0(4) N(1)-Mn(1)-N(5) 87.5(5) N(3)-Mn(1)-N(5) 98.5(5) N(7)-Mn(1)-N(5) 143.7(4) C(5)-N(1)-C(1) 123.7(16)

	N(5)-C(30)	1.318(18)	C(5)-N(1)-Mn(1)	114.0(11)
	N(6)-C(31)	1.345(19)	C(1)-N(1)-Mn(1)	122.1(12)
	N(6)-C(35)	1.362(16)	C(10)-N(2)-C(6)	119.3(14)
	N(7)-C(36)	1.371(16)	C(10)-N(2)-Mn(1)	122.6(11)
	N(7)-C(40)	1.415(19)	C(6)-N(2)-Mn(1)	118.0(11)
	N(8)-C(44)	1.279(18)	C(11)-N(3)-C(15)	114.4(15)
	N(8)-C(47)	1.464(9)	C(11)-N(3)-Mn(1)	121.6(12)
	N(8)-C(49)	1.471(9)	C(15)-N(3)-Mn(1)	124.0(11)
	Mn(1)-N(6)	2.190(12)	C(19)-N(4)-C(22)	118.7(15)
	Mn(1)-N(2)	2.214(12)	C(19)-N(4)-C(24)	123.8(14)
	Mn(1)-N(1)	2.215(14)	C(22)-N(4)-C(24)	114.3(15)
	Mn(1)-N(3)	2.223(13)	C(26)-N(5)-C(30)	120.0(15)
	Mn(1)-N(7)	2.257(13)	C(26)-N(5)-Mn(1)	123.4(12)
	Mn(1)-N(5)	2.293(13)	C(30)-N(5)-Mn(1)	116.6(11)
	N(1)-C(5)	1.323(18)	C(31)-N(6)-C(35)	121.2(14)
	N(1)-C(1)	1.337(18)	C(31)-N(6)-Mn(1)	117.8(10)
	N(2)-C(10)	1.326(18)	C(35)-N(6)-Mn(1)	121.0(11)
	N(2)-C(6)	1.366(19)	C(36)-N(7)-C(40)	119.4(13)
	N(3)-C(11)	1.274(17)	C(36)-N(7)-Mn(1)	118.9(11)
	N(3)-C(15)	1.406(19)	C(40)-N(7)-Mn(1)	121.7(10)
	N(4)-C(19)	1.400(18)	C(44)-N(8)-C(47)	122.9(15)
	N(4)-C(22)	1.476(9)	C(44)-N(8)-C(49)	119.3(14)
	N(4)-C(24)	1.482(9)	C(47)-N(8)-C(49)	113.2(14)
	N(5)-C(26)	1.310(17)	N(6)-C(35)-C(34)	116.6(15)
	N(5)-C(30)	1.318(18)	N(6)-C(35)-C(36)	115.5(14)
	N(6)-C(31)	1.345(19)	N(7)-C(36)-C(37)	113.7(15)
	N(6)-C(35)	1.362(16)	N(7)-C(36)-C(35)	114.1(14)
	N(7)-C(36)	1.371(16)	C(39)-C(40)-N(7)	123.0(15)
	N(7)-C(40)	1.415(19)	N(8)-C(44)-C(45)	124.4(15)
	N(8)-C(44)	1.279(18)	N(8)-C(44)-C(43)	126.0(17)
	N(8)-C(47)	1.464(9)	N(8)-C(47)-C(48)	112.2(13)
	N(8)-C(49)	1.471(9)	N(8)-C(49)-C(50)	111.8(15)

Table S4. Fluorescence quantum yield and two-photon absorption cross section of **MTP**.

Compounds	λ_{max}[a]	λ_{max}[b]	Φ[c]	δ[d]
MTP	362 nm	540 nm	0.397	164.7 GM

[a]The maximum absorption wavelength. [b]The maximum emission wavelength. [c]The Quantum yields. [d]The maximum two-photon absorption cross section values in GM (1 GM = 10^{-50} cm^4 s $photon^{-1}$).

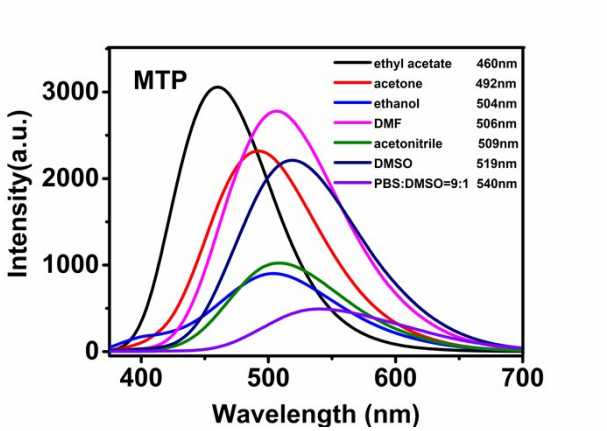


Fig. S5 One-photon excited fluorescence spectra of **MTP** in different solvents with c = 1.0×10^{-5} mol/L.

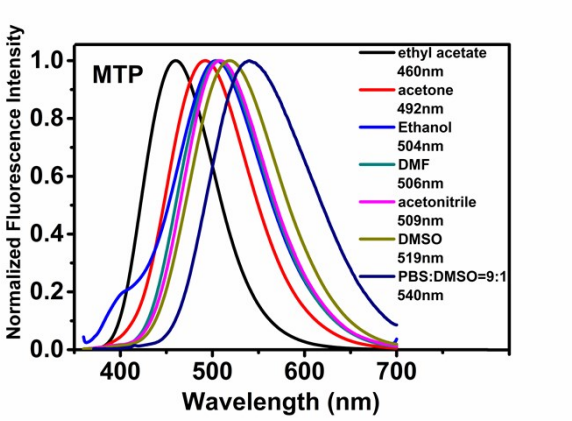


Fig. S6 The normalized one-photon excited fluorescence of the **MTP** in different solvents.

The effect of solvent on the emission spectra was further examined (Fig. S5) and the recorded fluorescence spectra clearly displayed positive solvatochromism (the emission peak in PBS was red shifted about 80 nm when compared with that in ethyl acetate) indicating the occurrence of polar excited states for the Mn^{II} complex. This result also indicated the emission to be a CT in nature.[8,9]

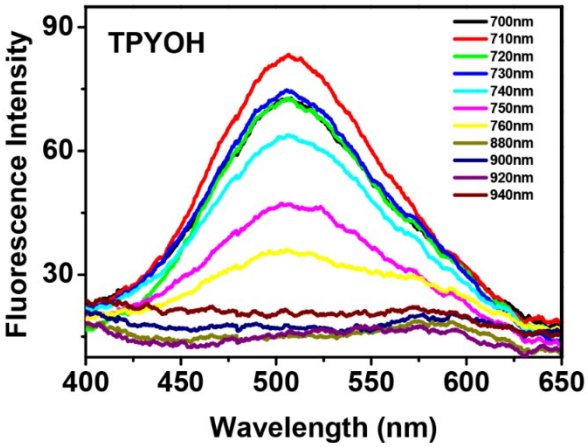


Fig. S7 Two-photon fluorescence spectra of TPYOH in DMSO by femtosecond laser pulses at 500 mW at different excitation wavelengths with c = 1.0×10^{-3} mol/L.

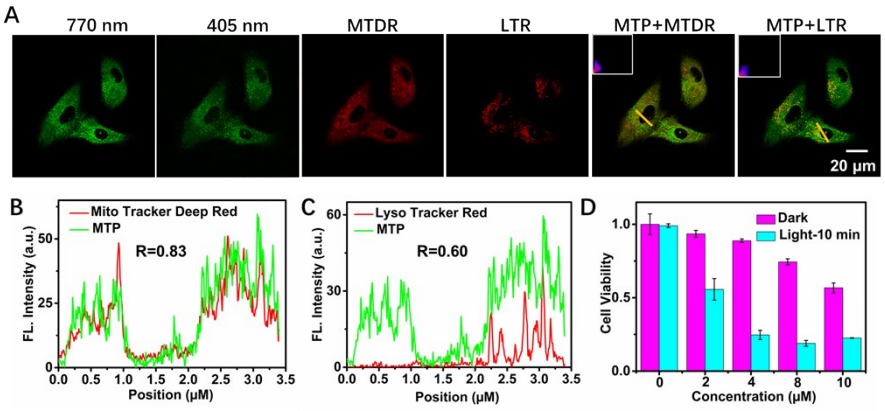


Fig. S8 (A) Co-localization experiments involving **MTP**, Mito Tracker Deep Red in A549 cells. The cells were incubated with Mito Tracker Deep Red (1 μM) for 30 min, Lyso Tracker Red (1 μM) for 30 min and incubated with **MTP** (5 μM) upon dark environment. Scale bars: 20 μm. (B) Correlation plot of **MTP** and MTDR intensities. (C) Correlation plot of **MTP** and LTR intensities. (D) Cell viability of A549 cells incubated with **MTP** in the dark or light irradiation.

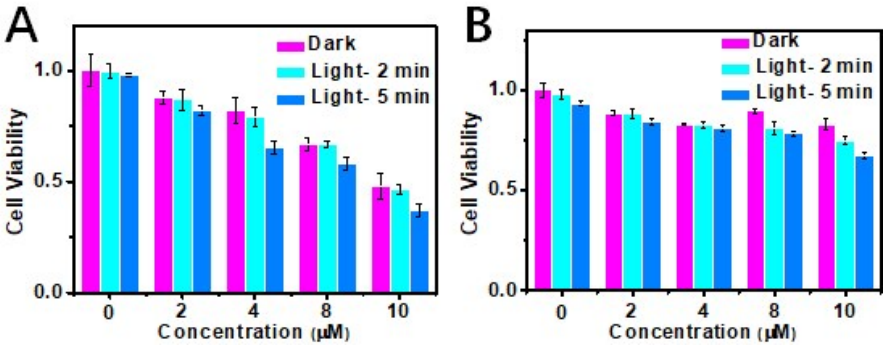


Fig. S9 (A) Cell viability of HeLa cells incubated with **MTP** in the dark or light irradiation. (B) Cell viability of LO2 cells incubated with **MTP** in the dark or light irradiation.

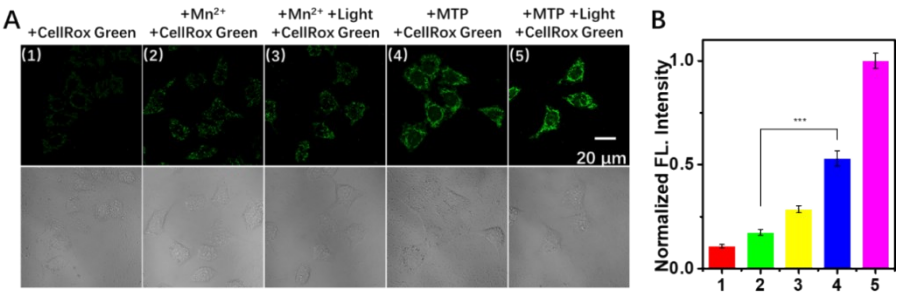


Fig. S10 (A) Confocal Images of the HeLa cells. (1) Incubated with 5 μM CellRox® Green for 30 min. (2) Incubated with 5 μM $Mn(NO_3)_2$ for 10 min and then incubated 5 μM CellRox® Green for 30 min. (3) Pretreated with 5 μM $Mn(NO_3)_2$ for 10 min and 1 min light irradiation, then incubated with 5 μM CellRox® Green for 30 min. (4) Incubated with 5 μM **MTP** for 10 min and then incubated 5 μM CellRox® Green. (5) Incubated with 5 μM **MTP** for 10 min and 1 min light irradiation, then incubated with 5 μM CellRox® Green. (B) Normalized fluorescence intensities of Fig. S10A (1-5). ***P < 0.001, n = 3, Student's t-test. Scale bar = 20 μm, PMT range: 500-540 nm, λ_{ex} = 488 nm. As shown in the Figure, HeLa cells incubated with 5 μM $Mn(NO_3)_2$ showed a weaker increase in ROS compared to cells incubated with **MTP**. This result validated our conjecture that **MTP** induced ROS instead of the Mn^{2+} under the dark conditions.

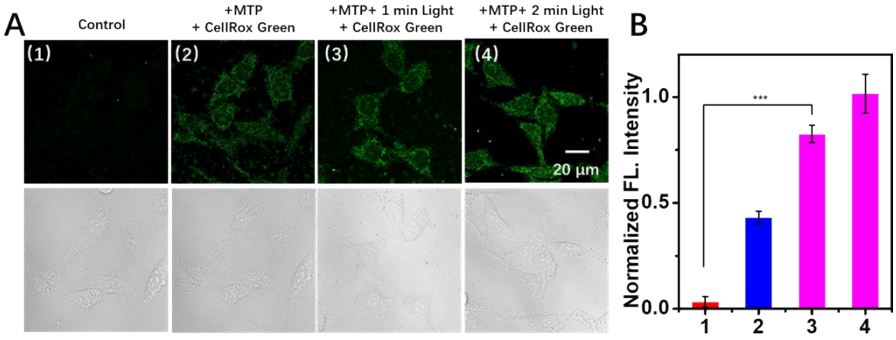


Fig. S11 Confocal Images of the HeLa cells. (1) With no pretreated. (2) Incubated with 5 μM **MTP** for 10 min and then incubated with 5 μM CellRox® Green for 30 min. (3) Incubated with 5 μM **MTP** for 10 min and 1 min light irradiation with 770 nm two-photon laser. Then incubated with 5 μM CellRox® Green for 30 min. (4) Incubated with 5 μM **MTP** for 10 min and then 2 min light irradiation with 770 nm two-photon laser. Then incubated with 5 μM CellRox® Green for 30 min. (B) Normalized fluorescence intensities of A (1-4). ***P < 0.001, n = 3, Student's t-test. Scale bar = 20 μm, PMT range: 500-540 nm, λ_{ex} = 488 nm.

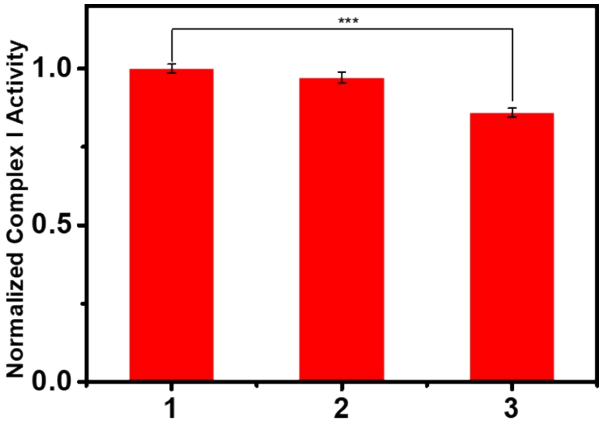


Fig. S12 The activity of Complex I was tested by mitochondrial respiratory chain complex enzyme I kit (Jinlin, China). (1) HeLa Cells incubated without **MTP.** (2) HeLa Cells incubated with 5 μM **MTP** for 30 min. (3) HeLa Cells incubated with 5 μM **MTP** for 30 min and 2 min light irradiation. ****P* < 0.001, n = 3, Student's t-test.

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